Four new *ent*-kaurene diterpene glucosides from *Mikania micrantha*

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ABSTRACT

Phytochemical study on the aerial parts of *Mikania micrantha* led to the isolation of four new *ent*-kaurene diterpene glucosides, β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**1**), β-D-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**2**), β-D-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**3**), β-D-glucopyranosyl-15α-(3-methyl-2-butenoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**4**), along with a known one, β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-*ent*-16-kauren-19-oate (**5**). Their structures were elucidated on the basis of extensive spectroscopic analysis. Compounds **1**–**4** are a group of C-9 hydroxylated *ent*-kaurene diterpene glucosides which is relatively rare in nature. These compounds selectively showed *in vitro* antibacterial activity against four assayed Gram-(+) and three Gram-(−) bacteria. In addition, the *in vitro* growth inhibitory activity of these compounds against human cancer cell lines Hela, A549, HepG-2 and MCF-7, were also tested.

Key words: *Mikania micrantha; ent*-Kaurane*;* Diterpene glucosides; Antibacterial activity; Cytotoxicity

1. Introduction

Mikania micrantha H. B. K. (Asteraceae), is a perennial creeping vine native to Central and South America. Away from its homeland, it grows very fast and has become an exotic invasive plant in Southeast Asia and South Asia, which has caused huge economic loss in agricultural and forestry production in the established areas (Feng et al., 2002; Zhang et al., 2004). In Mexico, *M. micrantha* has long been used as a traditional folk remedy for the treatment of skin diseases, snake bites and scorpion stings (Aguilar, 1994). Previous phytochemical studies have revealed some structurally diverse chemicals from this plant, including terpenoids, steroids, flavonoids and phenolic compounds, some of which showed important bioactivities (Boeker et al., 1987; But et al., 2009; Cuenca et al., 1988; Herz et al., 1975; Huang et al., 2004; Nicollier and Thompson, 1981; Ríos et al., 2014; Wei et al., 2004; Xu et al., 2013a, 2013b). As one part of our work on searching for potentially new and bioactive natural products from invasive plants in China (Luo et al., 2015; Ren et al., 2015; Yan et al., 2010; Zhang et al., 2014; Zhou et al., 2013), we carried out a phytochemical study on the aerial parts of *M. micrantha*, by which four new C9-hydroxylated *ent*-kaurene diterpene glucosides (**1**–**4**), along with a known diterpene glucoside (**5**) were obtained. In this paper, we describe the isolation and structure elucidation of these compounds, as well as their *in vitro* antibacterial and cytotoxic activity.

2. Results and discussion

Compound **1** was obtained as white amorphous powder with a molecular formula $C_{31}H_{48}O_{11}$ as determined by HR-ESI-MS data, *m/z* 631.2895 $[M + Cl]^-$ (calcd for

$C_{31}H_{48}O_{11}Cl^-$, 631.2891), which required eight degrees of unsaturation. The ^1H NMR spectrum (Table 1) showed signals of four tertiary methyls at δ_H 1.12 (3H, s, Me-20), 1.24 (3H, s, Me-18), 1.31 (3H, s, Me-4") and 1.31 (3H, s, Me-5"), an oxymethine at δ_H 6.02 (1H, s, H-15), and an exomethylene at δ_H 5.09 and 5.17 (1H each, s, H-17). The ^{13}C NMR spectrum (Table 2), coupled with HSQC analysis, indicated thirty-one carbons, including four methyls [δ_C 18.2 (C-20), 29.1 (C-18), 29.3 (C-4"), and 29.6 (C-5")], eleven methylenes, eight methines and eight quaternary carbons [including two oxygenated quaternary carbons at δ_C 70.4 (C-3") and 77.8 (C-9)], two carboxyl groups [δ_C 178.5 (C-19) and 172.9 (C-1")] and an exocyclic olefinic group [δ_C 110.6 (C-17) and 157.5 (C-16)]. The presence of a β-D-glucopyranosyl moiety in the molecule was suggested by the signals of δ_H 5.44 (1H, d, J = 8.0 Hz, H-1') and δ_C 62.4 (C-6'), 71.1 (C-4'), 74.0 (C-2'), 78.7 (C-3'), 78.7 (C-5'), and 95.6 (C-1'). Careful analysis of the NMR data indicated that compound **1** closely resembled β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-*ent*-16-kauren-19-oate (Xu et al., 2013a), a known *ent*-kaurene diterpene glucoside **5** which was also obtained in this study. The major difference pointed out by the NMR data was that the signals of the methine group at C-9 [δ_H 1.19 (1H, m, H-9); δ_C 54.5 (C-9)] in **5** were replaced by a hydroxylated quaternary carbon signal [δ_C 77.8 (C-9)] in **1**. These above findings led us to establish the structure of **1** as shown in Fig. 1. This assignment was consistent with the molecular formula and in accord with the observed HMBC correlations from δ_H 1.12 (H-20), 1.57 (H-12), 6.02 (H-15) to δ_C 77.8 (C-9). Besides, the observation of HMBC correlation from δ_H 5.44 (H-1') to δ_C 178.5 (C-19) (Fig. 2) verified the location

of the glucopyranosyl moiety at C-19. Moreover, the HMBC correlations from δ_H 1.31 (6H, s, H-4" and H-5") to δ_C 48.9 (C-2") and δ_H 2.48 and 2.51 (1H each, d, J = 14.1 Hz, H-2") to δ_C 172.9 (C-1") (Fig. 2) verified the presence of a 3-hydroxy-3-methylbutanoyloxy moiety (Xu et al., 2013a). Furthermore, the HMBC correlations from δ_H 6.02 (H-15) to C-9, C-13, C-14 and C-1" (Fig. 2) supported the location of the butanoyloxy moiety at C-15. The observed NOE correlation between H-15 and Me-18 further indicated the β-orientation of H-15. Thus, compound **1** was determined as β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate.

Compound **2** was also obtained as white amorphous powder. Its molecular formula $C_{31}H_{48}O_{10}$ was assigned based on the HR-ESI-MS data, *m/z* 615.2936 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941). The closely related NMR data of **2** with those of **1** (Tables 1 and 2) suggested that **2** was also an *ent*-kaurene diterpene glucoside. Further comparison of the NMR spectra showed that the signal for the hydroxylated quaternary carbon C-3" in **1** was absent in **2**. Instead, additional signals for a methine group [δ_H 2.09 (1H, m, H-3"), δ_C 27.0 (C-3")] were exhibited. These findings supported **2** having the same basic structure as **1**, with the only difference of the hydroxylated C-3" in **1** being replaced by a methine group in **2**. This deduction was consistent with the molecular formula and in accord with the change of the chemical shifts and coupling constants of CH_2-2", Me-4" and Me-5" as compared to those in **1** (see Tables 1 and 2), as well as supported well by the HMBC correlations from δ_H 0.98 (6H, d, J = 6.6 Hz, H-4" and H-5") to δ_C 44.8 (C-2") and δ_H 2.19 (2H, dd, J = 7.1,

1.6 Hz, H-2") to δ_C 174.6 (C-1") (Fig. 2). Therefore, compound **2** was elucidated as β-D-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate.

Compound **3** having a molecular formula $C_{31}H_{48}O_{10}$ the same as **2**, as deduced by HR-ESI-MS analysis, *m/z* 615.2942 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941). Detailed comparison revealed that the ^1H and ^{13}C NMR data (Tables 1 and 2) of **3** were closely related to those of **2**, except that the signals for the 3-methylbutanoyloxy moiety locating at C-15 in **2** being replaced by signals for a 2-methylbutanoyloxy moiety in **3**. These findings supported to establish **3** as a molecule closely related to **2** with the only difference of one methyl group locating at C-3" in **2** shifted to C-2" in **3**. This deduction was in complete accordance with the observed change of 1D NMR data from C-1" through C-5" (Tables 1 and 2) and further supported by the HMBC correlations from δ_H 1.14 (3H, d, J = 7.0 Hz, H-5") to δ_C 178.1 (C-1") and δ_H 0.93 (3H, t, J = 7.4 Hz, H-4") to δ_C 43.1 (C-2") (Fig. 2). Consequently, compound **3** was established as β-D-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate.

The HR-ESI-MS spectrum established the molecular formula of compound **4** as $C_{31}H_{46}O_{10}$, *m/z* 613.2791 $[M + Cl]^-$ (calcd for $C_{31}H_{46}O_{10}Cl^-$, 613.2785). By comparison, it was found that the ^1H and ^{13}C NMR data of **4** were similar to those of **2** except that the methylene group at C-2" and the methine group at C-3" in **2** were absent in **4** (see Tables 1 and 2). Instead, one singlet proton resonance at δ_H 5.65 and two carbon signals at δ_C 117.3 and 157.7 for a trisubstituted double bond (–CH(2")=C(3")–) were present. These findings supported to establish **4** as a molecule

closely related to **2** with the only difference of one double bond appearing between C-2" and C-3". This assignment was consistent with the molecular formula of **4** and well supported by HMBC correlations from δ_H 1.91 (3H, d, J = 1.0 Hz, H-4") and 2.16 (3H, d, J = 1.0 Hz, H-5") to δ_C 117.3 (C-2") and δ_H 5.65 (1H, br s, H-2") to δ_C 168.1 (C-1") (Fig. 2). Therefore, compound **4** was identified as β-D-glucopyranosyl-15α-(3-methyl-2-butenoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate.

The known compound **5** was identified as β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-*ent*-16-kauren-19-oate by comparison of their spectral data (^{1}H and ^{13}C-NMR and MS) to those reported in the literature (Xu et al., 2013a). Among these compounds, **1**–**4** are a group of C-9 hydroxylated *ent*-kaurene diterpene glucosides which is relatively rare in nature. By retrieving concerned literatures, we found that only a few C-9 hydroxylated *ent*-kaurene diterpene glucosides were reported (Ohtani et al., 1992; Richter et al., 1977; Shimizu et al., 1990; Takahashi et al., 2004; Tanaka et al., 1981; Tellez et al., 2004; Torrenegra et al., 1999).

The *in vitro* antibacterial activity of the five isolated compounds (**1**–**5**) against four Gram-(+) bacteria (*Bacillus subtilis*, *Curtobacterium flaccumfaciens*, *Bacillus cereus* and *Staphyloccocus aureus*) and three Gram-(–) bacteria (*Salmonella typhimurium*, *Pseudomonas solanacearum* and *Escherich coli*) were evaluated by a microdilution titer assay as previously described (Wang et al., 2014). Compounds **1**–**5** showed weak antibacterial activity against all the assayed bacteria.

Compounds **1**-**5** were also tested for their *in vitro* growth inhibitory activity against four human cancer cell lines, Hela, A549, HepG-2 and MCF-7, by the MTT

method (Fu et al., 2014). As shown in Table 3, Compounds **1-5** selectively exhibited moderate cytotoxicity against the four tested tumor cell lines with IC_{50} values from 25.86 to 66.26 μM, but their bioactivities were all weaker than the reference compound Adriamycin (IC_{50} 1.87~3.06 μM).

3. Experimental

3.1. General experimental procedures

High-resolution (HR) ESI-MS was measured on a Bruker Bio TOF ⅢQ spectrometer (Bruker Daltonics, USA). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX-500 NMR spectrometer (Bruker Biospin Gmbh, Rheistetten, Germany). Optical rotations were obtained on a Perkin-Elmer Model 341 polarimeter (Perkin-Elmer, Inc., Waltham, MA). UV spectra were acquired on a Perkin-Elmer Lambda 650 UV-vis spectrometer (Perkin-Elmer, Inc., Waltham, MA). Medium pressure liquid chromatography (MPLC) was carried out on a CXTH P3000 instrument (Beijing Chuang Xin Tong Heng Science and Technology Co., Ltd, Beijing, China) equipped with a UV 3000 UV-vis Detector and a C-18 column (50 μm, 50×500 mm). Preparative HPLC was performed with an HPLC system epuipped with a Shimadzu LC-6AD pump and a Shimadzu RID-10A refractive index detector using a Shim-pack PRC-ODS C-18 column (5 μm, 20×250 mm). Column chromatography (CC) was performed with silica gel (80-100 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Oppsala, Sweden). Analytical grade ethyl acetate, chloroform, methanol, petroleum ether (b.p. 60-90˚C), *n*-Butanol were purchased from Tianjin Fuyu Fine

Chemical Industry Co. (Tianjin, China). Thin-layer chromatography (TLC) was conducted on precoated silica gel plates (HSGF254, Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China) and spot detection was performed by spraying 10% H_2SO_4 in ethanol, followed by heating.

3.2. Plant material

The aerial parts of *M. micrantha* were collected from Guangzhou, China, in June 2012, identified by Prof. Hong-Feng Chen at South China Botanical Garden, the Chinese Academy of Sciences (CAS). A voucher specimen (No. 20120615) was deposited at the Laboratory of Bioorganic Chemistry of the South China Botanical Garden, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried aerial parts of *Mikania micrantha* (25 kg) were powdered and extracted three times with 95% EtOH at room temperature for three days each time. After concentration under vacuo, the EtOH extract was suspended with water and then sequentially extracted three times each with petroleum ether, EtOAc and *n*-Butanol. The EtOAc-soluble fraction (300 g) was subjected to silica gel column chromatography, eluted with $CHCl_3$/MeOH (from 100:0 to 0:100, v/v, each 21L) to afford E_1–E_{12}. Fraction E_9 (18.6 g), obtained on elution with $CHCl_3$/MeOH (90:10), was separated by MPLC using MeOH/H_2O (10:90-100:0) system at the flow rate of 10 mL/min to give $E_{9\text{-}1}$–$E_{9\text{-}30}$. Fraction $E_{9\text{-}17}$, obtained from the elution with MeOH/H_2O (60:40), was applied on Sephadex LH-20 CC with the elution of $CHCl_3$/MeOH (1:4, v/v), to give $E_{9\text{-}17\text{-}1}$–$E_{9\text{-}17\text{-}8}$. The fraction $E_{9\text{-}17\text{-}1}$ was further purified

by preparative HPLC with a Shim-pack PRC-ODS C-18 column (5 μm, 20×250 mm) using 22% acetonitrile in water (v/v) as mobile phase at the flow rate of 8 mL/min to obtain **1** (10 mg, t_R = 130 min). Fraction $E_{9\text{-}24}$, obtained from the elution with MeOH/H_2O (70:30), was subjected to Sephadex LH-20 CC with the elution of $CHCl_3$/MeOH (1:4, v/v), to give $E_{9\text{-}24\text{-}1}$–$E_{9\text{-}24\text{-}4}$. The fraction $E_{9\text{-}24\text{-}1}$ was further purified by preparative HPLC with a Shim-pack PRC-ODS C-18 column (5 μm, 20×250 mm), eluted with 32% acetonitrile in water (v/v) at the flow rate of 8 mL/min to obtain **4** (5 mg, t_R = 138 min), **3** (4 mg, t_R = 150 min), and **2** (5 mg, t_R = 168 min). Fraction $E_{9\text{-}29}$, obtained from the elution with MeOH/H_2O (85:15), was separated by Sephadex LH-20 CC which was eluted with methanol and then purified by HPLC using 65% methanol in water (v/v) as mobile phase at the flow rate of 10 mL/min to furnish compound **5** (40 mg, t_R = 81 min).

*3.4. Spectral data of compounds **1–4***

β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**1**): white amorphous powder; $[\alpha]_D^{20}$ -46.3 (*c* 0.87, CH_3OH); UV (CH_3OH) λ_{max} nm (log ε) 220 (2.83); HR-ESI-MS (neg.), *m/z* 631.2895 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{11}Cl^-$, 631.2891); The ^1H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD, see Tables 1 and 2.

β-D-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**2**): white amorphous powder; $[\alpha]_D^{20}$ -11.7 (*c* 0.90, CH_3OH); UV (CH_3OH) λ_{max} nm (log ε) 220 (3.28); HR-ESI-MS (neg.), *m/z* 615.2936 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941) ; The ^1H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD,

see Tables 1 and 2.

β-D-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**3**): white amorphous powder; $[\alpha]_D^{20}$ -38.5 (*c* 0.69, CH_3OH); UV (CH_3OH) λ_{max} nm (log ε) 220 (3.32); HR-ESI-MS (neg.), *m/z* 615.2942 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941); The ^1H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD, see Tables 1 and 2.

β-D-glucopyranosyl-15α-(3-methyl-2-butenoyloxy)-9β-hydroxy-*ent*-16-kauren-19-oate (**4**): white amorphous powder; $[\alpha]_D^{20}$ -42.0 (*c* 0.19, CH_3OH); UV (CH_3OH) λ_{max} nm (log ε) 220 (4.03); HR-ESI-MS (neg.), *m/z* 613.2791 $[M + Cl]^-$ (calcd for $C_{31}H_{46}O_{10}Cl^-$, 613.2785); The ^1H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD, see Tables 1 and 2.

3.5. Antibacterial activity assay

A total of seven microorganisms including four Gram-(+) bacteria (*Bacillus subtilis*, *Curtobacterium flaccumfaciens*, *Bacillus cereus* and *Staphyloccocus aureus*) and three Gram-(−) bacterial species (*Salmonella typhimurium*, *Pseudomonas solanacearum* and *Escherich coli*) were used in the bioassay. They were all obtained from the Microbial Culture Collection Center of Guangdong Institute of Microbiology (Guangzhou, China). Cultures of the bacteria were maintained in 20% glycerol-water medium and stored at -80˚C freezer. Resazurin and kanamycin sulfate were purchased from Sigma Chemical Co. (Sigma-Aldrich, St. Louis, MO, USA). This assay was carried out according to the procedures as previously described (Wang et al., 2014).

3.6. Cytotoxicity assay

Four assayed human cancer cell lines Hela, A549, HepG-2 and MCF-7 were all obtained from The Cell Bank of Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and adriamycin (ADM) were purchased from Sigma Chemical Co. (Sigma-Aldrich, St. Louis, MO, USA). This assay was performed following the procedures as previously described (Fu et al., 2014).

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Figure captions:

Fig.1. Chemical structures of compounds **1**–**5**

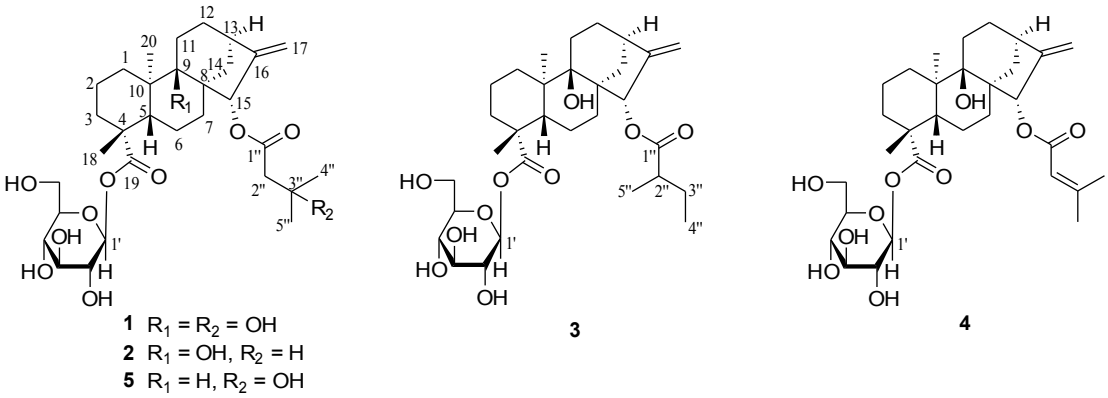

Fig.2. Selected HMBC correlations (arrows) of compounds **1**–**4**

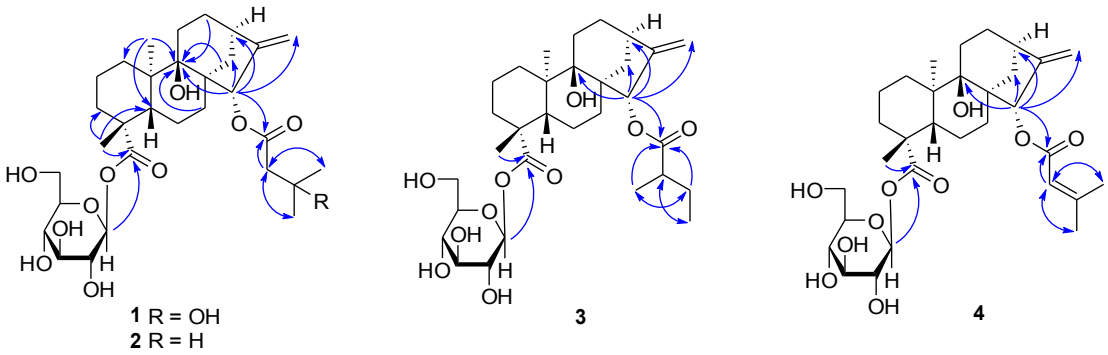

Table 1

^{1}H NMR（500 MHz）assignments [δ (ppm), J in Hz] of compounds **1**–**4** in CD_3OD

Position	**1**	**2**	**3**	**4**
1	1.51 (m)	1.51 (m)	1.51 (m)	1.51 (m)
	1.79 (m)	1.79 (m)	1.78 (m)	1.76 (m)
2	1.46 (m)	1.49 (m)	1.47 (m)	1.47 (m)
	1.95 (m)	1.97 (m)	1.95 (m)	1.94 (m)
3	1.06 (dd, 13.4, 3.9)	1.06 (dd, 13.3, 3.9)	1.06 (dd, 13.4, 4.0)	1.04 (dd, 13.4, 3.9)
	2.16 (br d, 13.4)	2.15 (m)	2.16 (br d, 13.4)	2.13 (m)
5	1.70 (dd, 12.7, 2.4)	1.70 (dd, 12.7, 2.4)	1.70 (m)	1.68 (dd, 12.8, 2.2)
6	1.86 (dd, 14.0, 2.4)	1.86 (dd, 14.0, 2.4)	1.86 (dd, 14.0, 2.5)	1.82 (m)
	2.05 (m)	2.03 (m)	2.04 (m)	2.00 (m)
7	1.51 (m)	1.49 (m)	1.52 (m)	1.47 (m)
	1.62 (m)	1.62 (m)	1.62 (m)	1.57 (m)
11	2.00 (m)	1.99 (m)	1.99 (m)	1.98 (m)
	2.02 (m)	2.02 (m)	2.02 (m)	2.00 (m)
12	1.57 (m)	1.56 (m)	1.57 (m)	1.57 (m)
	1.74 (m)	1.74 (m)	1.75 (m)	1.74 (m)
13	2.74 (br s)	2.73 (br s)	2.73 (br s)	2.72 (br s)
14	1.57 (m)	1.56 (m)	1.57 (m)	1.57 (m)
	2.27 (br d, 12.4)	2.27 (br d, 12.4)	2.27 (br d, 12.3)	2.25 (br d, 12.4)
15	6.02 (s)	5.99 (s)	5.98 (s)	6.00 (s)
17	5.09 (s)	5.08 (s)	5.07 (s)	5.05 (s)
	5.17 (s)	5.14 (s)	5.12 (s)	5.10 (s)
18	1.24 (s)	1.24 (s)	1.24 (s)	1.22 (s)
20	1.12 (s)	1.12 (s)	1.12 (s)	1.10 (s)
1'	5.44 (d, 8.0)	5.44 (d, 8.0)	5.43 (d, 8.0)	5.42 (d, 8.0)
2'	3.36-3.45 (m)	3.36-3.45 (m)	3.36-3.44 (m)	3.35-3.43 (m)
3'	3.36-3.45 (m)	3.36-3.45 (m)	3.36-3.44 (m)	3.35-3.43 (m)
4'	3.36-3.45 (m)	3.36-3.45 (m)	3.36-3.44 (m)	3.35-3.43 (m)
5'	3.36-3.45 (m)	3.36-3.45 (m)	3.36-3.44 (m)	3.35-3.43 (m)
6'	3.71 (dd, 11.9, 4.3)	3.71 (dd, 11.9, 3.9)	3.70 (dd, 11.9, 4.1)	3.69 (dd, 11.9, 4.2)
	3.85 (dd, 11.9, 1.2)	3.84 (dd, 11.9, 1.5)	3.84 (dd, 11.9, 1.6)	3.83 (dd, 11.9, 1.6)
2"	2.48 (d, 14.1)	2.19 (dd, 7.1, 1.6)	2.35 (m)	5.65 (br s)
	2.51 (d, 14.1)	2.19 (dd, 7.1, 1.6)		
3"		2.09 (m)	1.49 (m)	
			1.68 (m)	
4"	1.31 (s)	0.98 (d, 6.6)	0.93 (t, 7.4)	1.91 (d, 1.0)
5"	1.31 (s)	0.98 (d, 6.6)	1.14 (d, 7.0)	2.16 (d, 1.0)

Table 2

^{13}C NMR（125 MHz）assignments [δ (ppm)] of compounds **1**–**4** in CD_3OD

Position	**1**	**2**	**3**	**4**
1	33.3 (CH_2)	33.3 (CH_2)	33.3 (CH_2)	33.3 (CH_2)
2	20.1 (CH_2)	20.1 (CH_2)	20.1 (CH_2)	20.1 (CH_2)
3	38.7 (CH_2)	38.7 (CH_2)	38.7 (CH_2)	38.8 (CH_2)
4	45.2 (C)	45.2 (C)	45.2 (C)	45.2 (C)
5	51.2 (CH)	51.2 (CH)	51.2 (CH)	51.2 (CH)
6	22.0 (CH_2)	22.0 (CH_2)	22.0 (CH_2)	22.0 (CH_2)
7	31.1 (CH_2)	31.0 (CH_2)	31.2 (CH_2)	31.0 (CH_2)
8	54.0 (C)	54.0 (C)	54.1 (C)	54.1 (C)
9	77.8 (C)	77.8 (C)	77.8 (C)	77.9 (C)
10	45.7 (C)	45.7 (C)	45.7 (C)	45.7 (C)
11	29.5 (CH_2)	29.6 (CH_2)	29.6 (CH_2)	29.6 (CH_2)
12	35.0 (CH_2)	35.0 (CH_2)	35.0 (CH_2)	35.0 (CH_2)
13	42.6 (CH)	42.6 (CH)	42.7 (CH)	42.7 (CH)
14	38.5 (CH_2)	38.5 (CH_2)	38.6 (CH_2)	38.7 (CH_2)
15	80.9 (CH)	80.7 (CH)	80.6 (CH)	79.9 (CH)
16	157.5 (C)	157.7 (C)	157.8 (C)	157.9 (C)
17	110.6 (CH_2)	110.4 (CH_2)	110.2 (CH_2)	110.1 (CH_2)
18	29.1 (CH_3)	29.1 (CH_3)	29.1 (CH_3)	29.1 (CH_3)
19	178.5 (C)	178.5 (C)	178.5 (C)	178.6 (C)
20	18.2 (CH_3)	18.2 (CH_3)	18.2 (CH_3)	18.2 (CH_3)
1'	95.6 (CH)	95.6 (CH)	95.6 (CH)	95.6 (CH)
2'	74.0 (CH)	74.0 (CH)	74.1 (CH)	74.1 (CH)
3'	78.7 (CH)	78.7 (CH)	78.7 (CH)	78.7 (CH)
4'	71.1 (CH)	71.1 (CH)	71.1 (CH)	71.1 (CH)
5'	78.7 (CH)	78.7 (CH)	78.7 (CH)	78.7 (CH)
6'	62.4 (CH_2)	62.4 (CH_2)	62.4 (CH_2)	62.4 (CH_2)
1"	172.9 (C)	174.6 (C)	178.1 (C)	168.1 (C)
2"	48.9 (CH_2)	44.8 (CH_2)	43.1 (CH)	117.3 (CH)
3"	70.4 (C)	27.0 (CH)	27.8 (CH_2)	157.7 (C)
4"	29.3 (CH_3)	22.7 (CH_3)	12.1 (CH_3)	27.4 (CH_3)
5"	29.6 (CH_3)	22.8 (CH_3)	17.3 (CH_3)	20.4 (CH_3)

Table 3

Cytotoxicity of compounds **1**–**5** (IC_{50}, μM).

Cell lines	Compounds					
	1	**2**	**3**	**4**	**5**	Adriamycin
A549	49.66±1.03	25.86±2.69	34.48±1.88	39.47±1.28	60.34±2.32	2.68±0.17
HepG-2	65.09±1.46	66.26±2.37	60.02±1.26	47.13±0.94	58.97±1.08	2.12±0.23
MCF-7	56.81±0.98	41.91±1.88	48.03±1.09	52.81±1.39	62.38±2.35	3.06±0.12
Hela	45.21±0.89	37.09±2.35	39.64±1.68	51.46±2.06	52.81±1.31	1.87±0.92